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Boning up on telomerase

The expression of telomerase in bone marrow stromal cells brings cell-based bone tissue engineering one step closer to reality.

Rocky Tuan

One of the secrets of longer life is now manifest (for bone marrow stromal cells at least). Although expansion of postnatal bone marrow stromal cells has been possible for several years[1], until now the potential of these cells to differentiate into many different lineages has diminished over time, leading to senescence and death after no more than 30–40 population doublings. Two papers in this issue by Simonsen *et al.*[2] and Shi *et al.*[3] provide a means of extending the life-span of marrow stromal cells, with important implications for their application to cell-based bone tissue engineering.

The regeneration of diseased or damaged tissue is the principal goal of the emerging discipline of tissue engineering. A key requirement in tissue regeneration is the availability of the constituent cells; these may be differentiated cells that correspond to the cell types of the desired tissue, or progenitor cells that can be guided to differentiate into the appropriate cell type *ex vivo* or *in situ*. As the supply of differentiated cells is limited and their continued propagation *in vitro* often leads to dedifferentiation, multipotent progenitor cells are often considered more desirable for tissue engineering applications. For connective tissues, one of the most promising sources of progenitor cell types is the bone marrow stroma.

Beginning with pioneering work by Friedenstein (reviewed in ref. 4), marrow stromal cells have been shown to differentiate into cells of the adipogenic, chondrogenic, and osteogenic lineages. The marrow stromal cells are usually isolated on the basis of their ability to adhere to tissue culture substrate and form colonies in culture. Implantation studies using various animal models strongly suggest that these stromally derived cells are able to regenerate skeletal tissues, for example, in segmental bone defect sites.

In the new work, both research teams use the ectopic expression of telomerase to extend the life-span of human marrow stromal cells. The telomere length of chromosomes is maintained by a ribonuclear protein complex that includes an integral RNA, which acts as the telomeric template, and a catalytic subunit (hTERT) with reverse-transcriptase activity (reviewed in ref. 5). Cellular senescence is generally associated with reduced expression of hTERT. Interestingly, primary isolates of marrow stromal cells have no detectable hTERT activity, perhaps contributing to their limited replicative life-span *in vitro*. Both groups used retroviral transduction to achieve stable expression of hTERT in the marrow stromal cells. In both studies, growth arrest of control marrow stromal cells was evident after 26–30 population doublings, whereas the replicative potential of the hTERT-transduced cells remained unchanged for up to 80 (ref. 4) or 260 (ref. 3) population doublings. Notably, the cells retained the ability to undergo osteogenic, adipogenic, and chondrogenic differentiation *in vitro*.

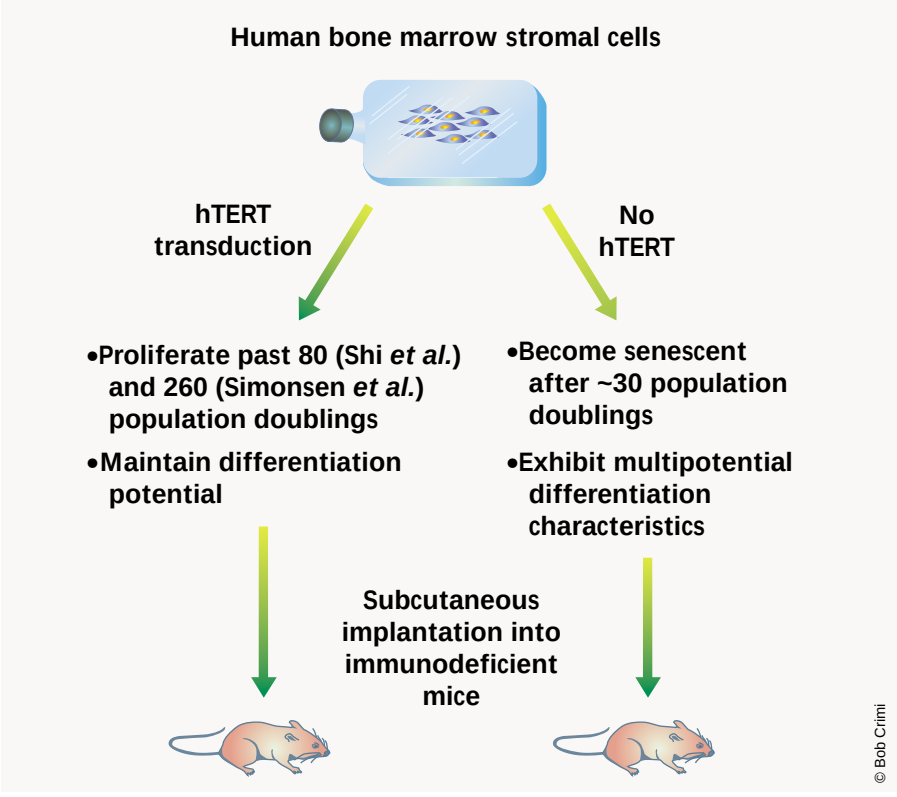

Figure 1. Telomerase expression extends the life-span and enhances the osteogenic potential of mesenchymal progenitor cells derived from human bone marrow stroma. Cells isolated from marrow stroma have multilineage differentiation potential and are considered a candidate cell source for mesenchymal tissue regeneration and engineering applications. They have a limited life-span, however, and senesce in culture. Retroviral transduction of human telomerase reverse transcriptase (hTERT) significantly extends their life-span, while maintaining their differentiation potential. Upon subcutaneous implantation in immunodeficient mice, the hTERT-transduced cells produce significantly more bony tissue than do the parental cells. The hTERT-transduced cells have a normal karyotype and are not tumorigenic.

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Even more interestingly, both groups observed that, when transplanted into immunodeficient mice, the hTERT-transduced cells generated more ectopic bone than did control cells. The regenerate bone exhibited all the cellular and matrix characteristics seen in normal control transplants, including lamellar bone covering the implant, bone-lining osteoblasts, encapsulated osteocytes, and fibrous tissues with associated hematopoietic marrow elements. The presence of standard matrix markers, such as collagen type I, was also detected. Histomorphometric analysis indicated 2–10-fold more bone formed by hTERT-transduced cells harvested at later cell passage (40 or more population doublings) than by control cells at earlier passage (15–20 population doublings).

The enhanced osteogenic potential of the hTERT-transduced marrow stromal cells was also observed *in vitro* when cells were maintained in osteogenic culture medium, as indicated by higher alkaline phosphatase activity and greater calcium deposition, and by expression of the osteoblast matrix protein osteocalcin and the osteoblast-associated transcription factor Cbfa1/Runx2. Shi *et al.* also report that hTERT maintained expression of the cell-surface marker STRO-1, which has been shown to be a characteristic of mesenchymal progenitor cells. Finally, the hTERT-transduced marrow stromal cells exhibited normal karyotype by G-banding analysis[3], and were non-tumorigenic as assayed by *in vitro* soft agar growth[4] and as indicated by their inability to form tumors upon subcutaneous implantation in immunodeficient or nude mice.

These two reports represent the first evaluation of the *in vivo* tissue-forming ability of marrow stromal cells ectopically expressing hTERT. The enhanced formation and normal morphology of the ectopically formed bone strongly suggest that hTERT-transduced marrow stromal cells represent a highly useful candidate cell source for bone tissue engineering. Given that the cells also maintain their adipogenic and chondrogenic abilities (although these studies did not analyze whether hTERT ectopic expression alters these abilities), they may be useful in engineering other connective tissues of mesenchymal origin.

These findings also raise interesting issues related to the biology of telomerase and of mesenchymal stem or progenitor cells. Because telomerase activity is associated with tumorigenesis (reviewed in ref. 5), additional measures will be necessary to ensure the safety of hTERT-transduced cells in tissue-engineering or tissue-regeneration applications. Several studies have reported that the number of marrow stromal progenitor cells declines as a function of age[6]. This is presumably due in part to the general senescence-associated decline in the replicative activity of these cells, perhaps related to decreased telomerase activity. It is thus noteworthy that both groups found that the parental marrow stromal cells had no detectable telomerase activity (which in part explains the dramatic life-span-extending activity of ectopic hTERT expression), even though the cells were derived from relatively young donors (a 33-year-old in Simenson *et al.* or 20–35-year-olds in Shi *et al.*). It would be interesting to carry out similar studies on cells from older individuals to determine the effect of hTERT expression in cells with a different replicative background.

The enhanced osteogenic potential of hTERT-transduced cells is of particular interest because it suggests that modulation of cellular replicative ability, and perhaps cell cycle characteristics, can actually influence the differentiation ability of osteoprogenitor cells. In general, cellular differentiation and cellular proliferation are thought to be mutually exclusive—in other words, differentiation represents a change, albeit a temporary one, in the cellular proliferation program. In the case of osteogenic differentiation, it has long been recognized that initial changes in chromatin structure and cell proliferation properties are crucial for regulating osteoblast-specific gene expression. It would be of interest to decipher how modifications of telomeric structures, perhaps involving altered nuclear matrix interactions, may enhance osteoblastic gene expression. In fact, it would be important to assess whether ectopic hTERT expression also alters adipogenesis and chondrogenesis, the two other major differentiation lineages for mesenchymal progenitor cells.

Finally, several recent studies have reported the identification and isolation of cells that have multilineage differentiation potential from various tissues, including skin[7], muscle[8], adipose tissue[9], and bone[10]. Although these cells are quite similar to marrow stromal cells in terms of their differentiation potential, they are also different in terms of their growth properties and perhaps their differentiation lineage preference. How mesenchymal progenitor cells derived from these other sources would respond to ectopic expression of hTERT, as compared with the response of the marrow stroma–derived cells, needs to be examined. Such studies will likely shed more light on the secret functions of cellular longevity.

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Sweet surrender to chemical genetics

Small molecules can extend the resolution of functional genomics beyond gene knockouts, allowing specific functions of a multifunctional protein to be modulated selectively.

Derek S. Tan

Gene knockouts are invaluable for studying the contribution of genes to phenotype, but they are rather blunt tools, particularly for dissecting the functions of proteins with more than one activity. The systematic use of small molecules to study protein function promises to serve as a powerful complement to other genomics strategies, especially since small molecules can be used to modulate individual functions of multifunctional proteins selectively. In a recent issue of *Nature*, Schreiber and colleagues[1] describe the latest progress toward this approach, identifying selective small-molecule probes that allow

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